EXPERIMENTS ON DISINFECTION OF WATER WITH ULTRA-VIOLET LIGHT, WITH A DISCUSSION OF THE LAWS OF DISINFECTION.*†

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INTRODUCTION.

The value of sunlight in human dwellings as an aid to healthy living has been recognized from involuntary human experience from time immemorial. The actual germicidal effect of light has been a subject of study ever since 1877, when Downes and Blunt,[2] in a classic series of experiments, demonstrated that light inhibited bacterial growth, and that the blue end of the solar spectrum was the more efficient for this purpose. A host of investigators studied the phenomenon in the eighties and nineties, some turning to the electric arc as a source of bacterial light, and a group, headed by Finsen, made the first application of the phenomenon in the treatment of certain forms of tuberculosis with the Finsen lamp. It was not until within a few years, however, that the invention of the quartz tube mercury arc lamp, and an awakened interest in water disinfection gave impetus to the study of water disinfection with ultra-violet light.

Curiously enough, the first work of this kind appears to have been done on milk. An anonymous communication to *Die Milchzeitung* (1909) states that Privatdozent Max Seiffert invented an apparatus for sterilizing milk with ultra-violet light in 1901. And Billon-Daguerre[3] (1909) in a communication to the Académie des Sciences, dated January 7, 1907, claims priority in the invention of a similar method.

The first published account of experiments in disinfection of water with ultra-violet light are by Courmont and Nogier[4] (1909). They have been closely followed by many others, including Billon-Daguerre[5] (1909), Henri, Helbronner, and de Reckling-

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[2] *Proc. Roy. Soc.*, 1877, 26, p. 488; 1878–79, 28, p. 199; 1886, 40, p. 14.

[3] *Compt. rend. Acad. d. Sci.*, 1909, 148, p. 542.

[4] *Ibid.*, 147, p. 523.

[5] *Ibid.*, 149, p. 810; 1910, 150, p. 479.

hauser[1] (1910), Gabriel-Vallet[2] (1910), Urbain, Scal, and Feige[3] (1910), Cernovodeanu and Henri[4] (1910), and Grimm and Weldert[5] (1911).

These observers have reported disinfection or sterilization of water with current consumptions varying from 75 to 900 kilowatt hours per million gallons. Others have studied the chemical effects of light on air and water, and still others have determined spectrographically which light waves are most effective in this respect. There has been a notable lack, however, of any satisfactory quantitative measure of the effect of varying such factors as distance, absorption, etc., and hence no working hypothesis suitable for the rational design of an apparatus for disinfecting water. It was with a view to determining whether or not these deficiencies could be supplied that the studies on which this paper is based were undertaken.

ON THE STANDARDIZATION OF DISINFECTANTS.

Previous attempts to estimate the disinfecting value of light under varying conditions have been based on the time required for complete sterilization of a given quantity of liquid. Such a measure, however, would be of no value for designing a disinfecting apparatus; for in such an apparatus the water would necessarily be moving, and could not be kept under constant conditions until sterilized. Moreover, anyone familiar with the typical curve of disinfection (see Fig. 1) must recognize that the intersection of its flattest part with the horizontal axis must be difficult to determine with any accuracy whatever, and that any method of standardization based on such a determination must be unsatisfactory, even if there is any simple relation between this time and the bactericidal efficiency of the disinfectant. Cernovodeanu and Henri[6] (1910) attempted to use this procedure, with the following results:

TABLE 1.

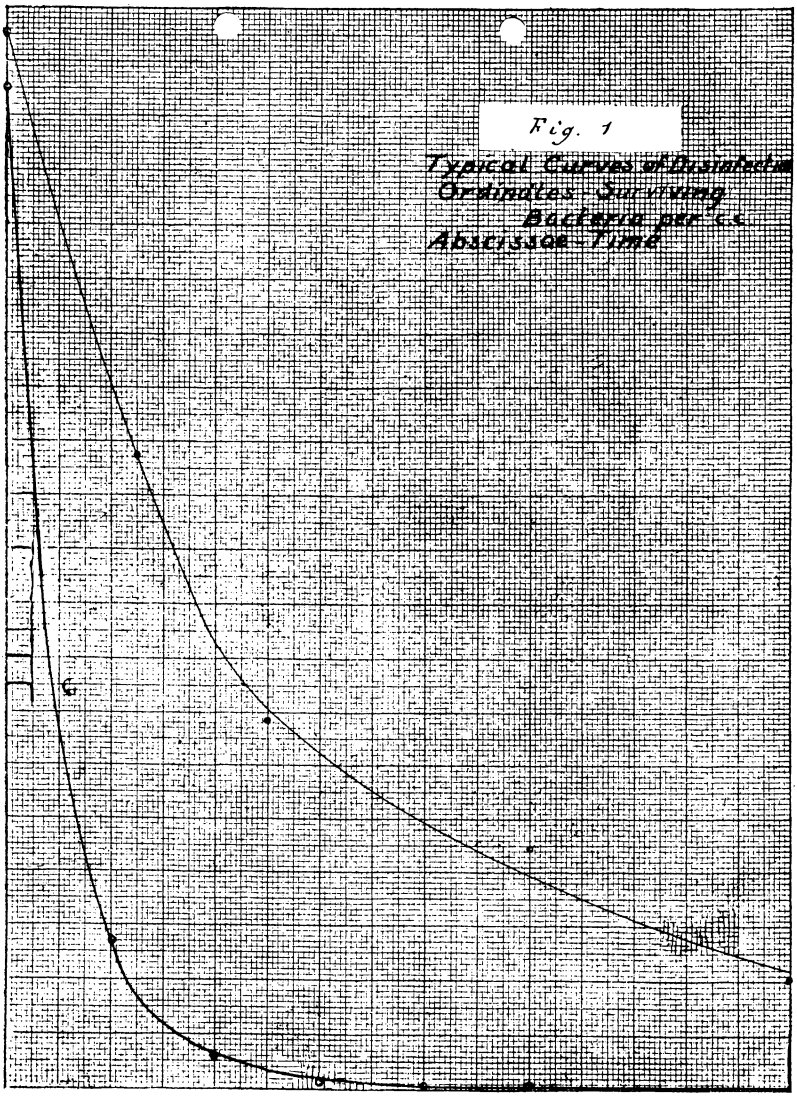
Distance	Time Necessary for Sterilization	
	110 v. Lamp	220 v. Lamp
cm.	Seconds	Seconds
60	300	30
40	180	15
20	20	4
10	4	1

[1] *Compt. rend. Acad. d. Sci.*, 1910, 150, p. 932; *ibid.*, 151, p. 677.

[2] *Ibid.*, p. 1076. [3] *Ibid.*, 151, p. 548; *ibid.*, p. 770. [4] *Ibid.*, 150, p. 52; *ibid.*, p. 549.

[5] *Sterilization von Wasser mittels ultravioletter Strahlen*, Berlin, 1911. [6] *Loc. cit.*

They concluded that there is no simple relation between time of sterilization and distance, and pointed out that the time varied more rapidly than the square of the distances.



More applicable to the practical problem would be a measure of the instantaneous effect—the rate of disinfection. And such a method has been proposed for chemical disinfectants as a result of

studies inspired by the observations that disinfection is always an orderly process with respect to time, and that the curve of disinfection is always of the same general type (see Fig. 1). In 1907 and 1908, Madsden and Nyman,[1] and Chick,[2] working simultaneously but independently, demonstrated that, in the disinfection of anthrax spores by chemicals, the logarithms of the numbers of living spores varied inversely as the time. Both concluded that, in the case of anthrax spores, the disinfection proceeds in the same manner as a monomolecular chemical reaction—in other words, that the rate of disinfection varies directly as the number of surviving spores. This theory has been developed and elaborated by Phelps[3] (1911) and supported by additional experimental evidence by Chick[4] (1910).

The mathematical form of this law is $\frac{dN}{dt}=kN$ where "N" is the number of living bacteria in a unit volume. Integrating between times t_1 and t_2, $\log \frac{N_2}{N_1}=k(t_2-t_1)$, and the accordance of the law with the facts will be represented by the degree of constancy of experimental determinations of $k=\frac{\log \frac{N_2}{N_1}}{t_2-t_1}$.

The experiments of Madsden and Nyman[5] (1907) on disinfection of anthrax spores with mercuric chloride, those of Chick[6] (1908, 1910) on disinfection of anthrax spores with phenol, and a few of her experiments with *B. typhosus*, *B. coli*, and other organisms, show remarkable agreement in the values of the constant obtained. But in the great majority of Chick's experiments on vegetative cells, in her analyses of those of Kronig and Paul,[7] and of Clark and Gage[8] (see Chick, *Jour. Hyg.*, 1910, 10, pp. 239–80), and in 10 of my own experiments, a gradual decrease in the constant, often of very great magnitude, was noted with increasing values of "t." As examples, I quote below values obtained from one of Chick's experiments, and from one of my own. Chick has used common

[1] *Ztschr. f. Hyg.*, 1907, 57, p. 388.
[2] *Jour. Hyg.*, 1908, 8, p. 92; p. 655.
[3] *Jour. Infect. Dis.*, 1911, 8, p. 1.
[4] *Jour. Hyg.*, 1910, 10, p. 237.
[5] *Loc. cit.*
[6] *Loc. cit.*
[7] *Ztschr. f. Hyg. u. Infektionskr.*, 1897, 25, p. 1.
[8] *Rep. State Bd. Health*, Massachusetts, 1903, 34, p. 268.

TABLE 2.

DATA TAKEN FROM CHICK (1908), TABLE IX, P. 108.

TIME (MINUTES) t_2	NUMBER BACTERIA IN ONE DROP OF DISINFECTION MIXTURE	VALUE OF $K=\frac{\log \frac{N_2}{N_1}}{t_2-t_1}$		
		$t_1=0$	$t_1=5$	$t_1=10$
0	25,250	....	....	....
0.5	540	3.74	....	....
1.0	305	1.92	0.50	....
2.1	97	1.15	0.47	0.45
3.1	50	0.87	0.40	0.37
4.1	24	0.74	0.38	0.36
5.2	10	0.65	0.37	0.35
7.0	2	0.57	0.36	0.36, etc.

or Briggs logarithms in all her work. I follow her in quoting her results, but in all my calculations, based on my own experiments, natural or Napierian logarithms are used.

TABLE 3.

DISINFECTION OF B. COLI WITH ULTRA-VIOLET LIGHT FROM MAGNETITE ARC AT 20 CM. DISTANCE.

TIME (SECONDS) t_2	NUMBER BACTERIA PER C.C.	VALUE OF $K=\frac{\log \frac{N_2}{N_1}}{t_2-t_1}$			
		$t_1=0$	$t_1=2$	$t_1=4$	$t_1=6$
0	185,000	....	....	....	.....
2	27,100	0.96	....	....	.....
4	5,950	0.86	0.76	....	.....
6	1,020	0.87	0.82	0.88	.....
8	990	0.65	0.55	0.45	0.015
10	880	0.54	0.43	0.32	0.037
15	420	0.41	0.32	0.24	0.099
20	211	0.34	0.27	0.21	0.11

It is significant that this change is always in one direction, and its regularity, when K values are plotted against time, suggests at once some controlling law. Chick recognized this and comments,[1] "The decrease in the value of K in the case of *B. paratyphosus* is a regular and orderly one. If values of K are plotted against numbers of surviving individuals, a continuous curve is obtained, showing that the value of K is altering in accordance with some law, and bears some relation to the number of surviving bacteria." In 1910 she made a similar comment,[2] and suggested that this deviation was connected in some way with variation among the bacteria,

[1] *Jour. Hyg.*, 1908, 8, p. 109. [2] *Ibid.*, 1910, 10, p. 282.

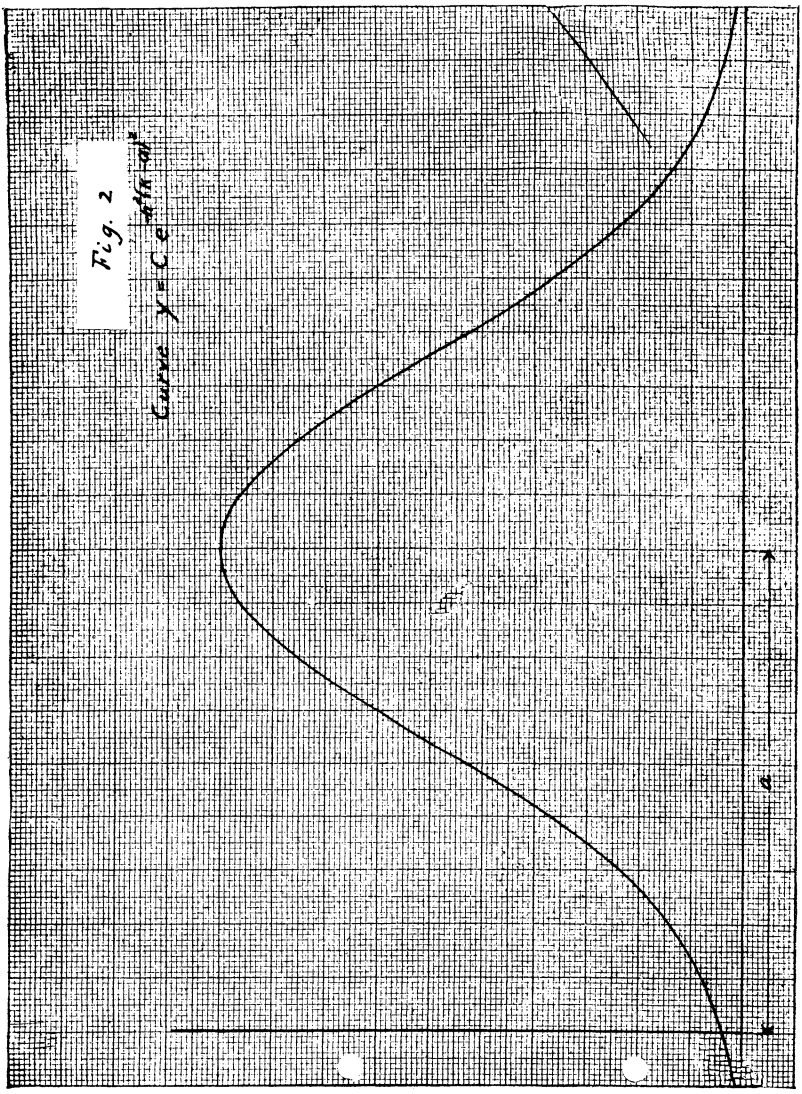
with respect to degree of possession of the property that causes disinfection to run logarithmically.

Following the line of this plausible suggestion, so much more in accordance with biometric experience than a theory based on equal possession of any natural property, I have developed the logarithmic theory of disinfection, assuming that any bacterial culture contains cells with varying K values, and that their distribution with respect to this property accords with biometric experience—namely, that there is a concentration of numbers about some mean or modal value, with smaller and smaller numbers departing extremely from this mode.

As a mathematical basis for this theory, two groups of curves were studied, both of which have been shown by biometricians to bear a strong general resemblance to the plotted curves of distribution of individuals with respect to biological characteristics. One, $y=Ce^{-h^2(k-a)^2}$ (see Fig. 2), is the "curve of error," representing the distribution of the errors of experimental observation. The other family, $y=C\left(\frac{k}{a}\right)^n e^{-h^2\left(\frac{k}{a}\right)^2}$ (see Fig. 3), is a group of which the member where $n=2$ is the curve representing the distribution of errors of mean square, and is the basis of Maxwell's kinetic theory of gases. In both cases, "a" is the value of "k" at the mode, and the area under any portion of the curve represents the number of organisms having "K" values between those corresponding to the limiting ordinates.

Without discussing the relative merits of these two sets of curves, or presenting the mathematical study that was made of both, it may be said that no analytical solution of either was found after several weeks' work. And while it was shown that solutions of the former by trial and error, and of the latter by a graphical method, are possible, neither could be worked out without a great amount of labor. Only a very few values were obtained in this way, not enough to determine whether or not such values would show any greater constancy than the "K" values previously discussed. It was demonstrated, however, that, in both cases, $\underset{t=0}{\text{limit}} \frac{\log \frac{B}{b}}{t} = Da$,

where "B" is the initial number in a unit volume, "b" is the number surviving in a unit volume after time "t," "a" is the modal



value of "K" at the beginning of the disinfection, and "D" is a constant for the culture independent of the kind or concentration of the disinfectant. (For this demonstration, one assumption was

necessary, viz.: that the relative proportion having "K" values greater than the mode will be the same, whatever the absolute value of "a.") It is therefore possible to obtain the ratio of "a" values (D canceling out) for different disinfectants, or for different conditions, by plotting $\frac{\log \frac{B}{b}}{t}$ against time, and extending the curves to intersect the axis $t=0$. As "a" represents the rate of action of the given disinfectant upon a specific organism, and not a composite value for a mixture of varying ones, it is reasonable that this ratio should represent a truer measure of the relative efficiency of the disinfectants, or of the same disinfectant under varying conditions, than the mean of the "K" values proposed by other investigators. And the constancy of "K" in the experiments on spores fits in with the theory through the reasonable explanation that there must be considerably less biological variation between the inert, resting cells, than between active, vegetative cells, carrying on a greater variety of processes and subjected to a greater number of selective influences.

EXPERIMENTAL.

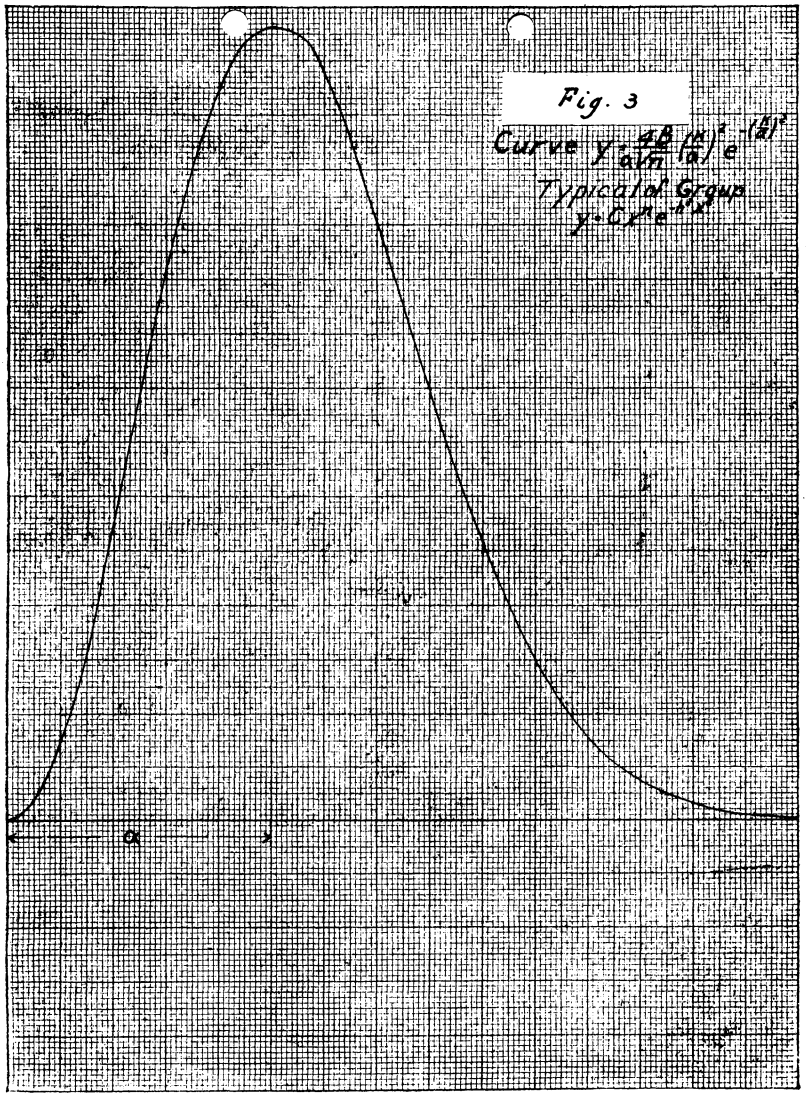
All the experiments made in this investigation were carried on with a laboratory stock culture of *B. coli*. A standard loopful was transferred every day to a tube of peptone, and a two-day old culture was used, except in one or two experiments. In general, the numbers in the two-day culture were reasonably constant, and between 100,000,000 and 200,000,000 per c.c., and a 1/1000 dilution in sterile water was used for the experiments.

Two sources of light were used. A magnetite arc without a globe, loaned by the Edison Electric Illuminating Co., of Boston, was connected with the 110 v., direct current circuit, and took about 6.6 amperes, on the average.

The other lamp was a Westinghouse-Cooper Hewitt quartz tube mercury lamp, made by the Westinghouse-Cooper Hewitt Co., of Paris, and loaned by the Cooper-Hewitt Co., of New York, for this investigation.

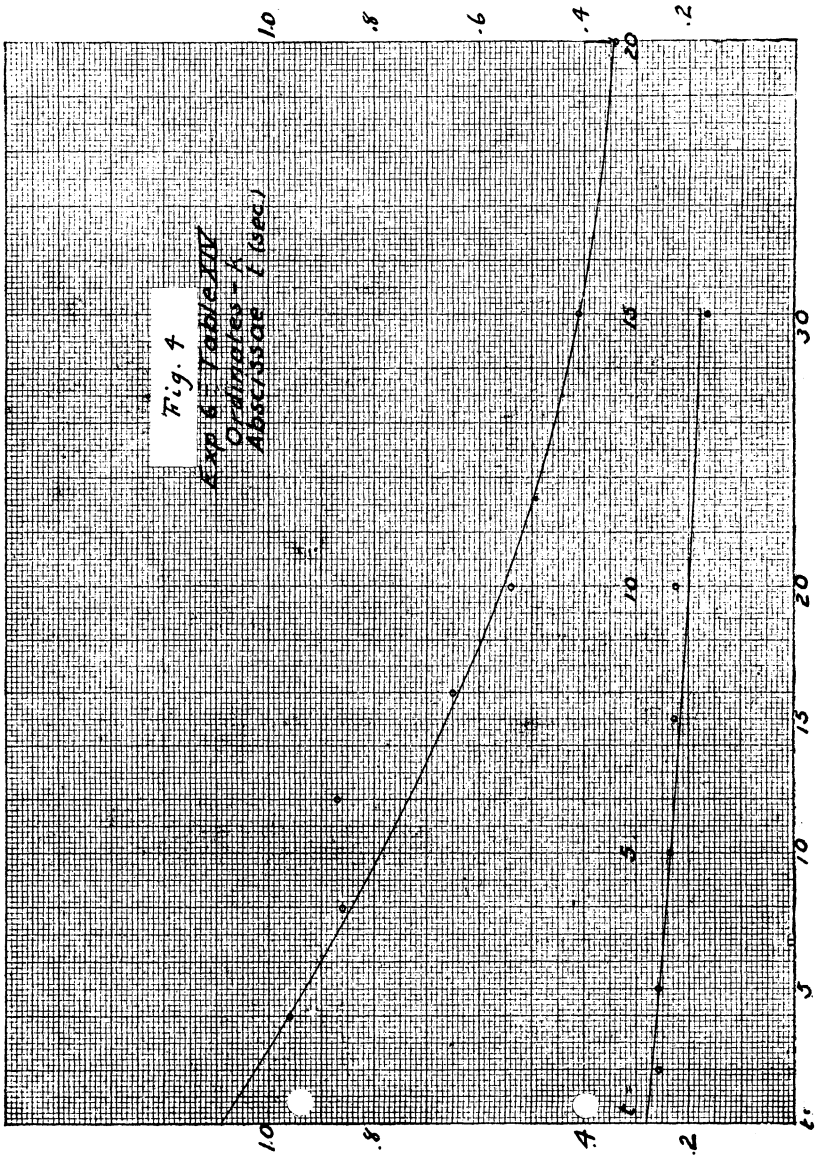
The lamp had a luminous tube about 12 cms. long and was connected with a 220-volt direct current circuit. The voltage was

cut down by interposing a resistance to 200 volts, and the lamp took, on the average, about 4.2 amperes.



The procedure finally adopted was as follows: About 1.2 c.c. of the 1/1000 dilution of the culture was pipetted into a quartz test tube, $\frac{3}{8}$ inches in diameter by about 3.5 inches long. The lamp

was lit, and the tube suspended in a ring in the proper position, and screened from the light with a slate screen. A test was started by



removing the slate screen quickly, and it was ended by throwing a switch and cutting off the lamp. Where it was impractical to

interpose the screen between the lamp and the tube, the tube was screened and held just above the ring. The test was then started by dropping the tube through the ring, and ended as before by cutting off the lamp. As soon as the lamp was cut off, the tube was removed, its mouth sterilized in a flame, and one c.c. of its contents transferred in a sterile pipette to the dilution water, or direct to a Petri dish if it was not to be diluted. Samples were plated with gelatin, incubated at 20° for 48 hours, and counted.

After several experiments, it was thought probable that growth in the diluted culture during the experiment might interfere with the work, and the plan was adopted of keeping it thereafter in a double walled vessel, packed with ice, so that the temperature was maintained at 8–10° C.

1. *On the effect of varying distance.*—Seven experiments were performed to study the effect of varying the distance in air from the light. Of these, three gave enough values of $\frac{\log \frac{B}{b}}{t}$, showing the usual orderly decrease to allow the construction of reasonably good curves. As an example of these curves, Fig. 4 shows those corresponding to Experiment 6 below. The results of these three experiments are given below:

TABLE 4.

Expt. 6, February 12, 1911. *B. coli*, two-day peptone. Samples rayed horizontally from magnetite arc, 110 v., 6.6 amp.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$k=\frac{\log \frac{B}{b}}{t}$
Distance 20 cm.				
0	175, 239, 140	3	185,000	...
2	238, 193, 292, 360	2	27,100	.96
4	61, 61, 53, 63	2	5,950	.86
6	135, 84, 68	1	1,020	.87
8	75, 93, 127, 102	1	990	.65
10	77, 96, 88, 91	1	880	.54
15	307, 540	0	420	.41
20	256, 158	0	210	.34
Distance 40 cm.				
0	168, 229, 251, 74	3	180,500	....
2	104, 115, 122, 90	3	108,000	.257
5	590, 487, 439, 465	2	49,500	.259
10	168, 184, 119, 165	2	17,300	.234
15	553, 524, 832, 816	1	6,810	.228
20	202, 265, 167, 180	1	2,035	.224
30	136, 132, 138, 118	1	1,310	.164

TABLE 5.

Expt. 7, February 13, 1911. *B. coli*, four-day peptone. Samples rayed horizontally from magnetite arc, 110 v., 6.6 amp.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K=\frac{\log \frac{B}{b}}{t}$
Distance 20 cm.				
0	309, 296, 266, 280	3	288,000	....
2	32, 36, 44, 34	3	36,500	1.03
4	74, 77, 87, 98	2	8,400	.88
6	23, 22, 28, 20	2	2,325	.80
8	72, 90, 88	1	833	.73
10	125, 126, 111, 95	1	1,140	.56
15	200, 428	0	314	.46
20	267	0	267	.35
Distance 40 cm.				
0	352, 341, 385, 411	3	372,000	.25
2	231, 242, 225, 200	3	224,500	.25
5	1060, 1060, 968, 952	2	101,000	.26
10	324, 325, 324	2	32,400	.24
15	1528, 1570, 1495, 1235	1	14,570	.22
20	456, 878, 750, 810	1	7,240	.20
30	290, 178, 171, 172	1	2,030	.17

TABLE 6.

Expt. 11, March 9, 1911. *B. coli*, two-day peptone. Samples rayed horizontally from mercury arc, 200 v., 4.2 amp.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K=\frac{\log \frac{B}{b}}{t}$
Distance 20 cm.				
0	322, 270, 276	3	289,300	....
2	18, 22	2	2,000	2.50
4	118, 172	1	1,450	1.33
8	377, 370, 340	0	362	.81
Distance 40 cm.				
0 taken same as above	.....	..	289,300	....
2	45, 57, 49	3	50,300	.87
5	156, 146	2	15,100	.59
10	615, 461	1	5,380	.40

Plotting $\frac{\log \frac{B}{b}}{t}$ against time, and extending the curves to meet the axis $t=0$, taking the intercept equal Da, we have, calling distance "L":

TABLE 7.

Experiment	L_1	Da_1	L_2	Da_2	$\left(\frac{L_2}{L_1}\right)^2$	$\frac{a_1}{a_2}$
	cm.		cm.			
6	20	1.08	40	.28	4	3.9
7	20	1.18	40	.29	4	4.1
11	20	4.70	40	1.17	4	4.0

In two other experiments it was possible to plot curves only by discarding one observation in each, which deviated extremely from the regularity indicated by the other observed values. In these cases the results were as follows:

TABLE 8.

Experiment	L_1	Da_1	L_2	Da_2	L_3	Da_3	$\left(\frac{L_2}{L_1}\right)^2$	$\frac{a_1}{a_2}$	$\left(\frac{L_3}{L_1}\right)^2$	$\frac{a_1}{a_3}$	$\left(\frac{L_3}{L_2}\right)^2$	$\frac{a_2}{a_3}$
	cm.											
8.....	40	.42	80	.10	..	....	4	4.2	..	...	..	...
9.....	20	.455	40	.195	80	.055	4	2.3	16	8.3	4	3.5

In the other two experiments, no plots of the results were possible.

2. *Relative efficiency of different sources of light.*—Two experiments were tried comparing the efficiency of the magnetite arc with that of the mercury arc. In neither case was it possible to construct "$K-t$" plots for both, and no quantitative relation was established. The experiment below, however, indicates that the mercury arc is many times as effective, although using only 1.2 times as much power:

TABLE 9.

Expt. 13, February 19, 1911. *B. coli*, two-day peptone. Samples rayed at 40 cm. horizontally from source of light.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K=\frac{\log \frac{B}{b}}{t}$
Magnetite arc				
0.....	277, 273, 314, 288	3	288,500	...
2.....	231, 202, 221	3	218,000	.14
5.....	131, 124	3	127,500	.16
20.....	125, 129, 132, 103	2	12,200	.16
Mercury arc 4.4 amp., 198 v.				
0.....	379, 400, 347, 380	3	376,500	....
2.....	239, 256	2	24,750	1.36
5.....	6, 12	2	900	1.21

3. *Absorption in water.*—Three experiments were made on the effect of interposing between the lamp and the sample various thicknesses of distilled water. A cylindrical galvanized iron tank, about 6 cm. in diameter and one meter long, and open at the top, was made, and a quartz plate about 1.1 mm. thick was set in one

end in a water-tight rubber joint, fastened between brass plates with four binding screws. The plates had a window about 2 mm. square cut out. Experiments were made by placing about 1.2 c.c. of the diluted culture in a quartz test-tube, and dropping the tube through a ring, so placed that the lower end of the tube passed through the open top of the tank into the water. The ring was at such a height that the tube was supported so as to bring the sample opposite the center of the quartz window. With this apparatus tubes could be exposed through any thickness of water up to one meter.

TABLE 10.

Expt. 20, April 1, 1911. *B. coli*, two-day peptone. Samples rayed horizontally opposite mercury arc lamp, 4.1 amp., 200 v.

Time (Sec.)	Counts	Dil.	Mean No. per c.c.	$K=\frac{\log \frac{B}{b}}{t}$
Distance 40 cm. in air.				
0	210, 225, 226, 262	3	231,000	...
2	114, 116	3	115,000	.35
4	55, 55	3	55,000	.36
6	225, 245	2	23,500	.38
Distance 22.5 cm., 2.5 in air and 20 cm. in distilled water.				
0 taken same as above			231,000	
2	155,171	3	163,000	...
5	129, 126	3	127,000	.17
10	95, 153	2		.12
	29, 21	3	18,700	.25
20	18, 10	2	1,400	.25
Distance 32.5 cm., 2.5 cm. in air and 30 cm. distilled water.				
0 taken same as above			231,000	
5	136, 157	3	146,500	.092
10	107, 141	3	127,000	.060
20	289,302	2	29,600	.103
Distance 42.5 cm., 2.5 cm. in air and 40 cm. in distilled water.				
0 taken same as above			231,000	
5	212, 207	3	209,500	.020
10	164, 144	3	154,000	.041
20	98, 85	3	91,500	.046
40	27, 45	3	36,000	.047

No satisfactory plots were possible, but as a rough measure of the order of magnitude of the relation, the means of these "K" values may be compared, assuming the values at the same distances in air to vary inversely as the square of the distance:

TABLE 11.

Distance (cm.)	Length Water Column (cm.)	Mean Value "K"	Calculated "K" at Same Distance in Air	Absorption (Percentage)
40	0	.36	.36	....
22.5	20	.20	1.14	82.5
32.5	30	.085	.55	84.5
42.5	40	.039	.32	88.0

The quantitative results cannot be supported in accordance with the method of treatment previously adopted; but they serve to indicate that absorption is greatest in the first layers, increasing slowly with increased thickness of water.

One experiment was tried on the absorption in Boston tap water, which had a color of about 0.4.

TABLE 12.

Expt. 21, April 2, 1911, *B. coli*, two-day peptone. Samples rayed at 22.5 cm. horizontal (2.5 cm. in air and 20 cm. tap water, color 0.4) from mercury arc lamp, 4.2 amp., 200 v.

Time	Counts	Dilution	Mean No. per c.c.
0	176, 192, 205, 182 177, 161, 120, 152	3	170,000
2	175, 194	3	184,000
5	208, 170	3	189,000
10	165, 165	3	165,000
20	164, 176	3	170,000

The absorption appears to have been complete, which is in accordance with the work of Courmont and Nogier,[1] (1909) on the impermeability to ultra-violet light of water containing colloidal matter.

CONCLUSIONS.

The method proposed for measuring the bactericidal effect of a disinfectant is a reasonable one, and slight support is given to it by the fact that, for those cases in which satisfactory plots were drawn, the calculated ratios of the rates of disinfection for the modal bacteria were closely inversely as the square of the distance, or as the intensity of light. It should be noted, however, that of the 10 experiments in which it was possible to plot "K-t" curves, five were preliminary experiments, and five were on the effect of varying distances in air. In the absorption experiments, in those on different sources of light, and in a few others, an irregular

[1] *Compt. rend. Acad. d. Sci.*, 1909, 149, p. 364.

variation in calculated "K" values, or lack of sufficient determinations, make it impossible to make satisfactory plots. Part of this may have been due to failure to make proper dilutions, through inability to predict the effect of these new conditions, and consequent loss of plates. But in some instances an ample number of points was determined, though varying so irregularly as to preclude plotting; and in a few cases an increase in the calculated values of $K=\frac{\log \frac{B}{b}}{t}$, instead of a decrease, was noted. No satisfactory explanation of these irregularities has suggested itself. Nevertheless, it is believed that the method is reasonable, and sufficiently novel and interesting to justify its presentation, not as a proved fact, but in the hope that it may lead to further study and experiment.

Granting the reasonableness of the method, some evidence is presented that the disinfecting power of ultra-violet light varies as the intensity of incident energy, or inversely as the square of the distance. And this has some further support in the fact that the absorption in water increases very slowly with increased distance, suggesting analogy to the law that the absorption of actinic energy varies directly as its intensity. If this is true, no analytical solution of the effect on water moving toward a light can be made, for no coefficient of absorption can be determined, except for monochromatic light. It would follow, then, that no rational design of an apparatus for disinfecting water can be made, but that the machine must be constructed in accordance with certain general principles, and modified to attain maximum efficiency by experiment.

The mercury arc lamp is more effective than the magnetite arc. There is reason to believe that its efficiency may be increased by varying the voltage, and the point of maximum efficiency would have to be determined experimentally.

Disinfection by ultra-violet light would be applicable to surface waters, containing vegetable coloring matter only if the color were first removed by coagulation or filtration.